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# Chronic increase of dietary L-tryptophan decreases gentle feather pecking behaviour

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**Abstract**

Many studies show the involvement of the serotonergic (5-HT) system in the performance of abnormal behaviour in both human and animals. Recently, we showed that acute reduction of 5-HT turnover in the forebrain, increased gentle and severe feather pecking behaviour in chicks from a high (HFP) and low feather pecking (LFP) line of laying hens, suggesting that the performance of feather pecking behaviour involves low 5-HT neurotransmission.

In the present study, we postulated that if low 5-HT is causally underlying feather pecking, increasing 5-HT turnover in the forebrain will decrease the development and performance of feather pecking. Augmentation of 5-HT neurotransmission in the brain was induced by chronically increasing dietary levels of the essential amino acid L-tryptophan (TRP) from which 5-HT is synthesised. From the age of 34 days, LFP and HFP chicks were fed a diet containing 2% TRP, whereas control birds of both lines were continuously fed with the normal rearing feed (0.16% TRP). From 35 days of age, litter was removed from the pens (10 pens/line-treatment) and all chicks (10 chicks/pen) were housed on a slatted floor until the end of the experiment. At 49 days of age, feather pecking behaviour was studied for 30 min. At 50 days of age baseline corticosterone, TRP and other large amino acids (LNAAs) were measured in the blood plasma of decapitated chicks (10 chicks per line-treatment). Furthermore, plasma corticosterone and central 5-HT turnover levels in response to manual restraint (5 min) were determined (10 chicks/line-treatment).

For neither gentle nor severe feather pecking a significant line × treatment interaction was found. However, TRP treatment resulted in a significant [$P = 0.02$] overall decrease of the frequency of gentle feather pecking. For severe feather pecking a similar but not significant pattern was found. Significant line effects were found for gentle and severe feather pecking. HFP birds showed significantly higher levels of gentle and severe feather pecking behaviour than LFP birds [$P < 0.001$]. TRP treatment significantly increased the TRP/LNAA ratio in the plasma of the chicks. Furthermore, TRP treatment overall increased baseline and stress-induced levels of plasma corticosterone (although

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more pronounced in the LFP line). TRP supplementation significantly increased 5-HT turnover in the hippocampus and archistriatum and tended to do so in the remainder of the forebrain.

The results confirm our hypothesis that feather pecking behaviour is triggered by low serotonergic neurotransmission, as increasing serotonergic tone, by increasing dietary TRP, decreases gentle feather pecking behaviour.


*Keywords:* Chicken feather pecking; Serotonin; L-Tryptophan

## 1. Introduction

Feather pecking in laying hens, an abnormal form of allopecking, is causing welfare problems in current poultry farming. Expressed in a more gentle, stereotypic form of pecking, feather pecking is abnormal, but not very harmful. The more severe, also rather compulsive form, feather pulling, ultimately results in injuries, leading to cannibalism and death. The causation of feather pecking is multifactorial(Hughes and Duncan, 1972), with interactions between animal characteristics (e.g. genetic factors) and environmental factors that have been proven hard to fathom.

Recently(van Hierden et al., 2004), we found evidence for a causal role of the serotonergic (5-HT) system in the performance of feather pecking behaviour. In a study with high (HFP) and low feather pecking (LFP) chicks, acute reduction of 5-HT turnover in the forebrain, increased gentle and severe feather pecking behaviour, suggesting that the performance of feather pecking behaviour involves low 5-HT neurotransmission in the forebrain. We postulated that if low 5-HT is causally underlying feather pecking behaviour, increasing 5-HT turnover in the forebrain should decrease the development and performance of feather pecking.

An effective tool for increasing 5-HT neurotransmission in the brain is increasing dietary levels of the essential amino acid L-tryptophan (TRP) from which 5-HT is synthesised (Fernstrom, 1983). For access into the brain, TRP competes with other large neutral amino acids (LNAA; i.e. tyrosine, phenylalanine, leucine, isoleucine, valine), as all LNAAs depend on the same carrier for transport across the blood–brain-barrier (Markus et al., 2000). An increase in the ratio of plasma TRP to the sum of the other LNAAs (TRP/ΣLNAAs), gives TRP an advantage in the competition for access into the brain (Markus et al., 2000). TRP hydroxylase, the rate-limiting enzyme on the pathway from TRP to serotonin is normally about half-saturated with TRP (Young and Leyton, 2002). Thus, elevated dietary intake of TRP, resulting in an increased plasma TRP/LNAA ratio, increases brain levels of TRP and elevates rates of 5-HT synthesis and metabolism (Johnston et al., 1990; Lepage et al., 2002).

TRP has been given to both man and animal, in disorders where a low level of serotonin has been suggested to be of etiological significance, for instance, depression (Sandyk, 1992), aggression (Shea et al., 1990; Winberg et al., 2001) and obsessive compulsive disorders (McDougle et al., 1999; Weld et al., 1998; Young and Leyton, 2002). In Rhesus monkeys, with a history of compulsive self-injurious behaviour, 3 weeks of dietary TRP supplementation, significantly increased 5-HIAA in the cerebrospinal fluid and significantly

decreased the duration of self-biting (Weld et al., 1998). In a study by Savory et al. (1999), dietary supplementation with TRP in growing bantams, resulted in a suppression of pecking damage with the higher (22.6 g/kg diet) dose, compared to the control (2.6 g/kg diet) dose, at 4 and 6 weeks of age. This lower level of pecking damage is very likely to be the result of a lower level of severe feather pecking behaviour. In that experiment, bantams were reared on a wire mesh floor, a condition known to increase feather pecking (Blokhuis, 1989).

From the available data it can be postulated that increasing the dietary TRP level in the feed of HFP and LFP birds, increases the level of central serotonin turnover and attenuates the development of feather pecking. To test this hypothesis, in the present study the effect of an increased dietary TRP level on the performance of feather pecking in HFP and LFP chicks was investigated. The dose of dietary TRP (1.6 g/kg versus 21 g/kg diet) used in this study, was chosen on the basis of results of studies from Savory et al. (1999), Rosebrough (1996) and Shea et al. (1990).

## 2. Methods

### *2.1. Birds and housing*

In this study 200 White Leghorn chicks were used: 100 LFP and 100 HFP chicks (for line specifications see Korte et al., 1997). All birds were female and non-beaktrimmed. Chicks arrived on the day of hatching and were kept in groups of 10 animals per line (20 groups per line) and housed in pens (0.75 m $\times$ 1.0 m) with woodshavings. The pens were placed in two identical climate controlled rooms. Individual pens were visually isolated by hardboard partitions. Four of the 10 birds per pen were individually marked with a waterproof marker.

The environmental temperature was lowered from 34 °C on day 1 to 18 °C at 45 days of age onwards. On days 1 and 2 of age the chicks received 24 h of light. From 3 days to 35 days onwards the light regime decreased from 18 h to 10 h light period.

All birds had access to three drinking cups and one square feeding trough placed along one of the walls of the pen. Water and feed (mash) were provided ad libitum.

### *2.2. Experimental design and treatment diets*

From day 1 till day 34 of age, all chicks received a standard rearing feed, containing 1.6 g TRP per kg feed (0.14% TRP is recommended by NRC, 1994). From the age of 34 days, half of the HFP and LFP birds, were fed a diet containing 2% TRP, which corresponds with $\pm$21 g TRP per kg diet. The other half of the birds were continuously fed the normal rearing feed (1.6 g/kg diet). There were 10 pens per line-treatment combination.

The LFP and HFP chicks were raised in litter floor pens (with woodshavings) until 35 days of age. The floor of the pen consisted of a slatted floor, on which cardboard was placed. A thick layer of woodshavings was applied onto the cardboard. At 35 days of age the cardboard with the layer of woodshavings was removed from the pens, with the aim to induce higher levels of gentle feather pecking or more severe levels of feather pecking behaviour (Blokhuis, 1989).

Table 1
Ethogram showing the behavioural measurements in (posture either standing or sitting)

| Behaviour | Definition |
|---|---|
| Frequencies | |
| Gentle feather pecking | Mild pecking at the feathers of conspecific, generally performed in multiple bouts (single pecks are counted as one occurrence) |
| Severe feather pecking | Vigorous pecking/pulling/pinching at the feathers of conspecific (single pecks are counted as one occurrence) |
| Duration | |
| Feeding | Pecking at food in trough |
| Foraging | Pecking at the litter and scratching (separately scored as ground scratching) or moving with the head in a lower position than the rump |
| Preening | Preening behaviour as described by Kruijt (1964): e.g. autopecking, nibbling, stroking, combing, head-rubbing |
| Walking | Walking, running, jumping or flying (it may be accompanied by wing-flapping) |
| Resting | Sitting or standing inactive (no movement of the legs) |

### 2.3. Behavioural observations

At 49 days of age, each pen was recorded on videotape for a period of 30 min. Afterwards, two of the four coloured birds per pen were randomly chosen and their behaviour was scored continuously for 30 min per bird using The Observer® 4.0 software program (Noldus, Wageningen, The Netherlands). Frequency and duration of the behavioural elements scored are described in Table 1.

### 2.4. Physiological and neurobiological measurements

The two observed coloured birds were used for physiological and neurobiological measurements at the age of 50 days. One of the observed birds per pen, was killed by rapid decapitation, upon removal from the pen, for baseline corticosterone, TRP and LNAAs measurements in the blood plasma. The other observed bird was removed from the pen, and manually restrained for 5 min (i.e. placed on its side). Subsequently, these restrained chicks were decapitated and their blood and brains were collected, for corticosterone and serotonin turnover measurements, respectively.

Trunk blood of both birds was collected in chilled (0 °C) lithium–heparin-coated centrifuge tubes, for either baseline or stress-induced plasma corticosterone measurements. Blood was centrifuged for 10 min at 3000 rpm at a temperature of 4 °C. Plasma samples for corticosterone analysis were stored at 4 °C in the presence of 0.1% (w/v) sodium azide. Corticosterone concentrations were determined in unextracted, enzymatically pretreated plasma (DELFIA), as described earlier (de Jong et al., 2001). The detection range of the corticosterone assay was 0.2–44 ng/ml.

Levels of TRP and Phenylanaline in the bloodplasma were analysed using deproteinization with sheep serum albumin (SSA). Analyses were performed with RP-HPLC: Alltima C18 column, sodiumacetate/methanol as eluents, pH 6.0. Detection with UV-detection at 207 nm. Plasma concentrations of the other LNAAs were measured by HPLC as described by de Jonge and Breuer (1994).

The brains of the restrained birds were removed within one minute after decapitation and immediately frozen in a dry ice precooled tube containing *n*-heptane and stored at −70 °C until the assays were performed. For the assay three brain sections were used: the hippocampus, the archistriatal complex (i.e. an amygdala like structure in birds, Kuenzel and Masson, 1988) and remainder of the forebrain. The brain sections were used for the measurement of serotonin (5-hydroxytryptamine; 5-HT) and the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA). Previously, it has been shown that 5-HT turnover is indicated by the 5-HIAA/5-HT ratio (Korte-Bouws et al., 1996).

The tissue samples were homogenised in 0.1 M perchloric acid and subsequently centrifuged at 14,000 rpm. The supernatant (50 μl) was injected onto a reverse-phase/ion pair HPLC set-up with electrochemical detection for the measurement of 5-HT and 5-HIAA. The chromatographic system consisted of an Pharmacia LKB HPLC pump 2158 (Kyoto, Japan), a Gilson 231 sample injector, an Antec Decade potentiostate (Antec Leyden, Leiden, The Netherlands) with a glassy carbon cell operated at +500 mV versus Ag/AgCl and a column (150 mm × 4.6 mm i.d.) packed with Supelcosil LC-18-DB, 3 μm particle size. The mobile phase consisted of sodium acetate (4.1 g/l), EDTA (0.15 g/l), sodium octane–sulfonic acid (0.175 g/l), tetramethylammonium (0.15 g/l), methanol (12%, v/v), pH 4.1. The flow rate was set at 1 ml/min.

### 2.5. Statistical analysis

Pens were considered to be the experimental units and the means of the two observed animals per pen were analysed. Durations of behaviours (i.e. percentages) were analysed with a logistic regression model with main effects and interactions for experimental factors for line (HFP or LFP) and treatment (0 or 50 mg per kg bodyweight), i.e. $\log(P/100-P)$ was expressed as a sum of main effects and interactions, where $P$ is the expected percentage. The model comprised a multiplicative dispersion factor relative to the binomial variance function, i.e. the variance was assumed to be proportional to $P^*(100-P)$. Frequency of feather pecking was analysed with a log linear model with main effects and interaction for line and treatment, i.e. $\log(m)$ is expressed as a sum of main effects and interactions, where $m$ is the expected mean. The model comprised a multiplicative dispersion factor relative to the variance under a Poisson distribution, i.e. the variance was assumed to be proportional to the mean $m$.

Estimation of durations and frequencies was performed by maximum quasi-likelihood. The dispersion parameter was estimated from Pearson's chi-square statistic. Significance tests were performed by referring the log quasi-likelihood ratio to an $F$-distribution (with 'residual degrees of freedom' corresponding to Pearson's chi-square statistic). Details may be found in McCullagh and Nelder (1989). Significance tests for feather pecking as a binary variable were based on the maximum likelihood ratio test.

Corticosterone, 5-HIAA/5-HT ratio and TRP/LNAA data were analysed with a mixed analysis of variance model with main effect and interactions for the factors line and treatment. For the analyses a common $F$-test was used. Residuals were checked for normal distribution and homogeneity of variances. Data showing heterogeneity, i.e. an increased variance with increasing mean, were logarithmically transformed and reanalysed.

Differences were considered significant if $P < 0.05$. For all calculations GenStat® 6 (2002) was used.

## 3. Results

### 3.1. Behavioural observations

Fig. 1 shows the results of the behavioural observations at 7 weeks of age.

For neither gentle (Fig. 1A) nor severe feather pecking behaviour (Fig. 1B) a significant line × treatment interaction was found (Table 2). For gentle feather pecking, a significant overall treatment effect ($F_{1,75} = 5.72$; $P = 0.02$) was found. For severe feather pecking no significant overall treatment effect was found ($F_{1,75} = 2.32$; $P = 0.13$). Fig. 1A shows that TRP-treated HFP birds showed significantly ($P = 0.03$) less gentle feather pecking than control HFP birds. For severe feather pecking a similar but not significant ($P = 0.19$) pattern was found (Fig. 1B). Significant overall line effects were found for gentle ($F_{1,75} = 35.15$; $P < 0.001$) and severe feather pecking ($F_{1,75} = 27.54$; $P < 0.001$). HFP birds showed significantly higher levels of gentle and severe feather pecking behaviour than LFP birds (Table 2).

For duration of foraging behaviour no significant line × treatment interaction was found. A trend for a treatment effect ($F_{1,75} = 3.15$; $P = 0.08$) and a significant effect of line were found ($F_{1,75} = 9.93$; $P < 0.001$). LFP birds spent more time foraging than HFP birds. Fig. 1C shows that LFP control birds significantly ($P = 0.04$) spent more time foraging than TRP-treated LFP birds.

For feeding behaviour a significant line × treatment interaction was found ($F_{1,74} = 5.40$; $P = 0.02$). Furthermore, significant effects of line ($F_{1,75} = 11.21$; $P < 0.001$) and treatment ($F_{1,75} = 30.20$; $P < 0.001$) were found (Table 2). Fig. 1D shows that in both lines TRP treatment significantly increases the duration of feeding behaviour, but more pronounced in the HFP line.

For preening behaviour no line × treatment effect was found, however a significant line effect ($F_{1,75} = 3.93$; $P = 0.05$) and treatment effect ($F_{1,75} = 9.09$; $P < 0.001$) were found. Fig. 1E shows that TRP-treated HFP birds spent significantly ($P = 0.004$) less time preening than control HFP birds.

For the duration of walking, a significant line × treatment effect was found ($F_{1,74} = 4.73$; $P = 0.03$). No significant overall line or treatment effect were found. Fig. 1F shows that

Table 2
Effects of line and treatment on behavioural observations of LFP and HFP chicks

| | Line | | Treatment | | Line × treatment | *P*-values[a] | |
|---|---|---|---|---|---|---|---|
| | LFP | HFP | Control | TRP | | Line | Treatment |
| Gentle FP | 18.55 ± 9.05 | 184.18 ± 29.0 | 137.70 ± 24.7 | 65.00 ± 17.8 | ns | *** | * |
| Severe FP | 2.08 ± 1.25 | 28.46 ± 4.29 | 16.18 ± 3.49 | 9.36 ± 2.78 | ns | *** | ns |
| Foraging (%) | 5.58 ± 0.64 | 3.05 ± 0.49 | 5.03 ± 0.60 | 3.60 ± 0.53 | ns | *** | # |
| Feeding (%) | 51.63 ± 3.26 | 36.09 ± 3.24 | 30.89 ± 3.09 | 56.83 ± 3.40 | * | *** | *** |
| Preening (%) | 9.61 ± 1.47 | 14.16 ± 1.77 | 15.38 ± 1.81 | 8.39 ± 1.43 | ns | * | *** |
| Walking (%) | 9.42 ± 0.82 | 7.96 ± 0.77 | 8.10 ± 0.76 | 9.29 ± 0.82 | * | ns | ns |
| Resting (%) | 23.50 ± 2.08 | 37.93 ± 2.42 | 40.22 ± 2.42 | 21.22 ± 2.08 | * | *** | *** |

[a] Significant effect: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, #$0.05 < P < 0.10$, ns: non significant.

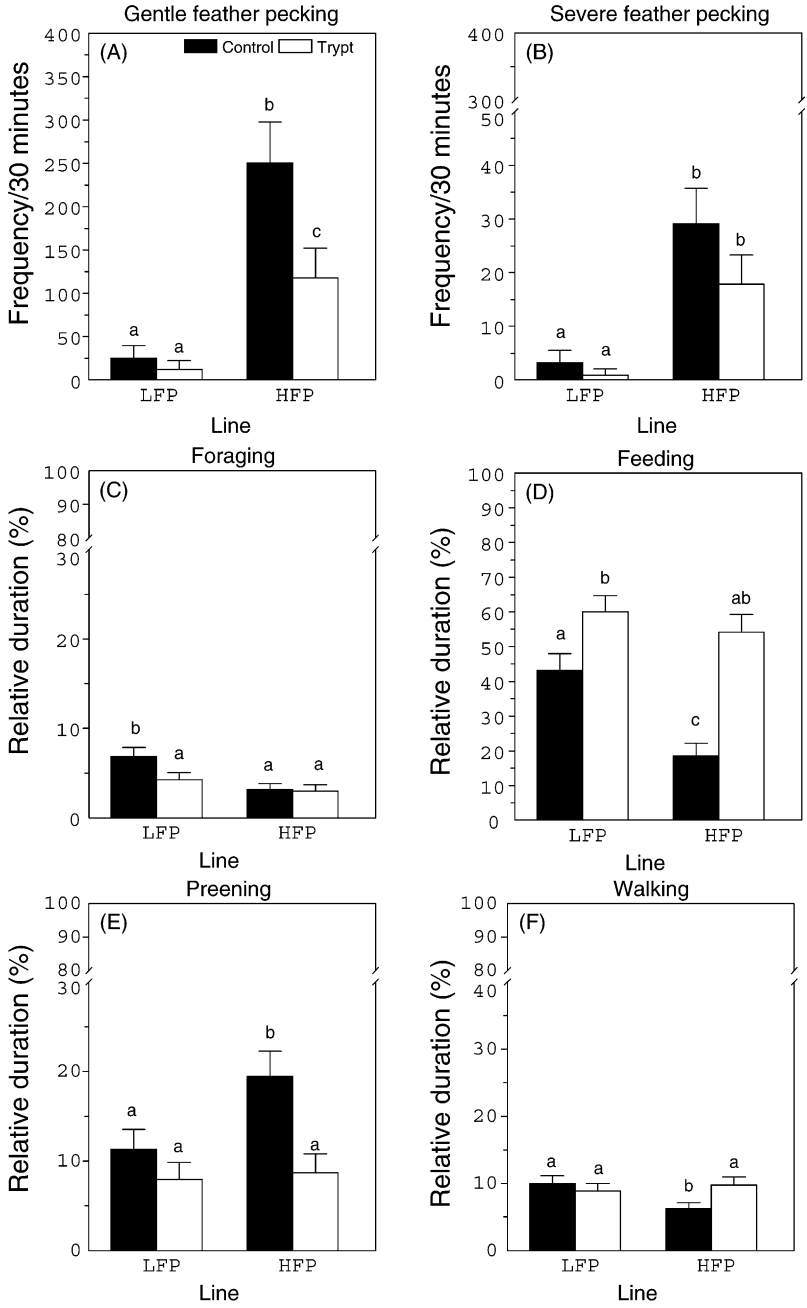


Fig. 1. Frequency or relative duration (percentage of the total observation time) of the behaviour of control and TRP-treated LFP and HFP chicks (49 days of age). Levels expressed as mean ± S.E.M. Means without a common superscript differ ($P < 0.10$).

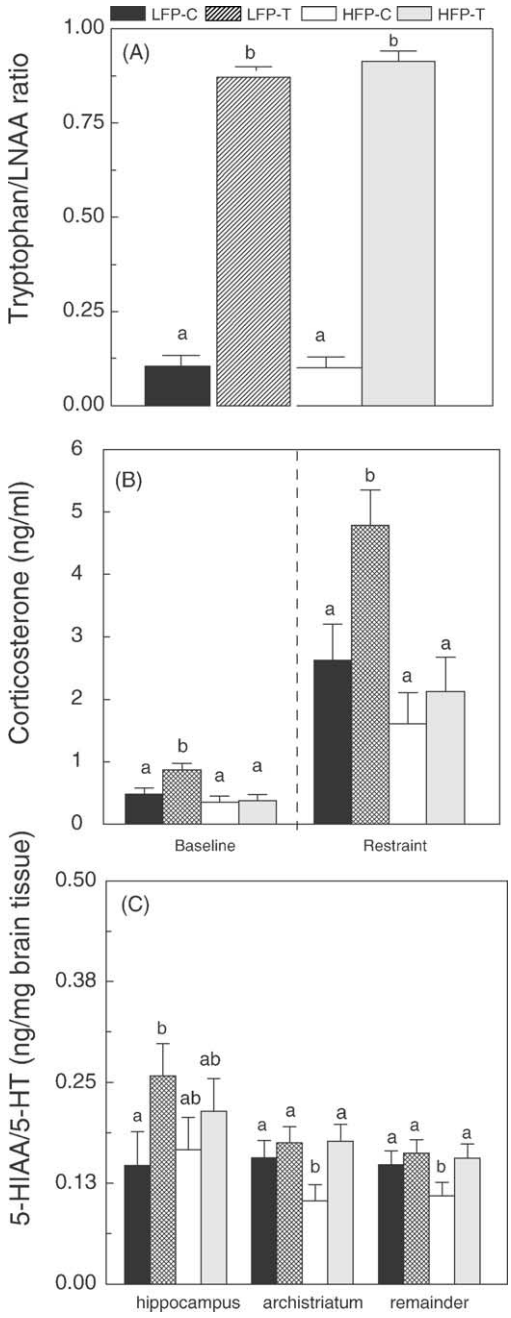


Fig. 2. Plasma levels of the TRP/LNAA ratio (A), baseline and restraint-induced corticosterone (B), and forebrain levels of 5-HT turnover (5-HIAA/5-HT) after manual restraint; LFP-C: LFP-control, LFP-T: LFP-tryptophan, HFP-C: HFP-control, HFP-T: HFP-tryptophan. Levels expressed as mean ± S.E.M. Means without a common superscript differ ($P < 0.10$).

TRP-treated HFP birds spent significantly ($P = 0.02$) more time walking than control HFP birds.

For resting behaviour a significant line × treatment interaction ($F_{1,74} = 5.02$; $P = 0.03$), line ($F_{1,75} = 22.28$; $P < 0.001$) and treatment ($F_{1,75} = 22.88$; $P < 0.001$) effects were found. TRP treatment decreased the duration of resting in both lines, but more pronounced in the HFP line (52.16 ± 0.04 versus 23.33 ± 0.03) than in the LFP line (23.33 ± 0.03 versus 18.73 ± 0.03).

Further results of the behavioural elements are shown in Table 2.

### 3.2. Corticosterone, TRP/LNAA, and serotonin measurements

For the TRP/LNAA ratio (Fig. 2A) no significant line × treatment interaction and line effect were found. TRP treatment however significantly increased ($F_{1,36} = 747.62$; $P < 0.001$) the TRP/LNAA ratio in the plasma of LFP and HFP chicks. Further results of the LNAAs are presented in Table 3.

For baseline corticosterone levels a trend for a line × treatment interaction was found ($F_{1,35} = 3.43$; $P = 0.07$). A significant treatment ($F_{1,36} = 3.92$; $P = 0.05$) and line effect ($F_{1,36} = 8.90$; $P = 0.005$) were found for baseline plasma corticosterone levels (Table 3). Fig. 2B shows that TRP-treated LFP chicks showed significantly higher baseline corticosterone levels compared to control LFP birds ($P = 0.01$).

For corticosterone levels after manual restraint (Fig. 2B), no significant line × treatment interaction was found. Both treatment ($F_{1,36} = 5.10$; $P = 0.03$) and line ($F_{1,36} = 10.41$; $P = 0.003$) effects were significant. Fig. 2B shows that stress-induced corticosterone levels were also significantly higher in the TRP-treated LFP chicks compared to control LFP birds ($P = 0.01$).

5-HT turnover levels (5-HIAA/5-HT) in the different brain regions are presented in Table 3 and Fig. 2C. For none of the brain regions significant line × treatment interactions or line effects were found. TRP supplementation however significantly increased 5-HT turnover in the hippocampus ($F_{1,36} = 3.85$; $P = 0.05$) and archistriatum ($F_{1,36} = 4.98$; $P = 0.03$) and tended to do so in the remainder of the forebrain ($F_{1,36} = 3.12$; $P = 0.09$). Fig. 2C shows that TRP treatment in LFP chicks significantly ($P = 0.05$) increased stress-induced 5-HT turnover levels in the hippocampus compared to control LFP chicks. For the HFP birds, no significant effect of TRP treatment on 5-HT turnover in the hippocampus was found. However, TRP treatment significantly increased 5-HT turnover in the archistriatum ($P = 0.01$) and the remainder of the forebrain ($P = 0.05$) in this line, whereas no significant effects were found for the LFP line in these brain regions.

## 4. Discussion

In the present study, the effect of TRP supplementation on the development of feather pecking behaviour was studied in HFP and LFP birds. The principal finding was that a dose of 21 g TRP/kg diet significantly reduced gentle feather pecking behaviour and increased the duration of feeding behaviour.

Table 3
Effects of line and treatment on [TRP/LNAA], plasma levels of [Cort-baseline] and [Cort-stress] (ng/ml), and brain levels of [5-HIAA] (ng/mg brain tissue), [5-HT] and [5-HIAA]/[5-HT] after manual restraint

| | Line | | Treatment | | Line × treatment | *P*-values[a] | |
|---|---|---|---|---|---|---|---|
| | LFP | HFP | Control | TRP | | Line | Treatment |
| TRP | 587.10 ± 26.2 | 596.50 ± 26.4 | 121.60 ± 11.9 | 1062.0 ± 35.2 | ns | ns | *** |
| Tyrosine | 248.00 ± 8.05 | 203.50 ± 7.30 | 216.75 ± 7.53 | 234.75 ± 7.84 | ns | *** | ns |
| Phenylalanine | 142.18 ± 3.71 | 130.98 ± 3.56 | 150.74 ± 3.82 | 122.42 ± 3.44 | ns | * | *** |
| Leucine | 307.78 ± 9.69 | 320.08 ± 9.88 | 320.11 ± 9.88 | 307.75 ± 9.69 | ns | ns | ns |
| Isoleucine | 159.58 ± 5.52 | 157.75 ± 5.48 | 154.42 ± 5.43 | 162.92 ± 5.57 | ns | ns | ns |
| Valine | 357.10 ± 10.6 | 351.60 ± 10.5 | 348.90 ± 10.5 | 359.80 ± 10.6 | ns | ns | ns |
| $5\text{-HIAA}_{hippo}$ | 0.20 ± 0.04 | 0.20 ± 0.04 | 0.13 ± 0.04 | 0.27 ± 0.04 | ns | ns | ** |
| $5\text{-HT}_{hippo}$ | 0.92 ± 0.04 | 1.00 ± 0.05 | 0.81 ± 0.04 | 1.11 ± 0.04 | ns | ns | *** |
| $5\text{-HIAA}_{arch}$ | 0.11 ± 0.01 | 0.08 ± 0.01 | 0.06 ± 0.01 | 0.13 ± 0.01 | ** | * | *** |
| $5\text{-HT}_{arch}$ | 0.69 ± 0.07 | 0.60 ± 0.07 | 0.55 ± 0.07 | 0.74 ± 0.07 | *** | ns | * |
| $5\text{-HIAA}_{rem}$ | 0.10 ± 0.01 | 0.10 ± 0.01 | 0.08 ± 0.01 | 0.12 ± 0.01 | ns | ns | *** |
| $5\text{-HT}_{rem}$ | 0.74 ± 0.02 | 0.68 ± 0.02 | 0.62 ± 0.02 | 0.79 ± 0.01 | ** | # | *** |
| [TRP/LNAA] | 0.49 ± 0.02 | 0.51 ± 0.02 | 0.10 ± 0.01 | 0.89 ± 0.02 | ns | ns | *** |
| [Cort-baseline] | 0.68 ± 0.09 | 0.45 ± 0.09 | 0.42 ± 0.09 | 0.70 ± 0.09 | ns | ** | * |
| [Cort-stress] | 3.70 ± 0.42 | 1.80 ± 0.37 | 2.17 ± 0.39 | 3.34 ± 0.37 | ns | ** | * |
| $[5\text{-HIAA}]/[5\text{-HT}]_{hippo}$ | 0.20 ± 0.03 | 0.19 ± 0.03 | 0.16 ± 0.02 | 0.24 ± 0.01 | ns | ns | * |
| $[5\text{-HIAA}]/[5\text{-HT}]_{arch}$ | 0.17 ± 0.01 | 0.14 ± 0.02 | 0.13 ± 0.01 | 0.18 ± 0.01 | ns | ns | * |
| $[5\text{-HIAA}]/[5\text{-HT}]_{rem}$ | 0.15 ± 0.01 | 0.13 ± 0.02 | 0.13 ± 0.01 | 0.16 ± 0.01 | ns | ns | # |

$N = 10$ birds per line-treatment combination; hippo: hippocampus, arch: archistriatum, rem: remainder of forebrain.

[a] Significant effect: $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, $^{\#}0.05 < P < 0.10$, ns: non significant.

### 4.1. Effect of TRP supplementation on physiological and neurobiological parameters

In both lines TRP treatment increased the TRP/LNAA ratio, which enabled an increase in 5-HT turnover in the forebrain of the chicks. These results confirm a study by Rosebrough (1996), that also showed TRP supplementation to be an excellent tool for increasing 5-HT turnover in the forebrain of chickens.

In the LFP line 5-HT turnover was increased in the hippocampus, whereas in the HFP line 5-HT turnover levels were increased in the archistriatum and the remainder of the forebrain. We tentatively suggest that differences in TRP hydroxylase activity between both lines, in the different brain regions, are responsible for the different levels of 5-HT turnover in response to TRP. In the LFP line, an increased TRP/LNAA ratio, was accompanied by an increase in both baseline and stress-induced plasma corticosterone levels, whereas in the HFP line corticosterone levels were not affected.

The increased adrenocortical (re)activity in the LFP line in response to TRP treatment, is in agreement with findings by Mench (1991) in broiler breeders. Mench (1991) found that an elevation in corticosterone, was modulated by dietary TRP. In mammals, 5-HT is known to be involved in the regulation of the HPA-axis (Chaouloff, 2000). Release of ACTH from the pituitary and glucocorticosteroids from the adrenal cortex is strongly and acutely stimulated by 5-HT via action mediated by 5-$HT_{1A}$ receptors in the hypothalamus (Fuller, 1992). An increase of corticosterone levels in the LFP, but not in the HFP line, is in agreement with previous findings of a higher adrenocortical (re)activity in the LFP line (Korte et al., 1997; van Hierden et al., 2002).

### 4.2. Effect of TRP supplementation on feather pecking behaviour

The results of this experiment support our hypothesis that increasing 5-HT neurotransmission in the forebrain of (HFP) chicks, by increasing dietary TRP, attenuates the development of feather pecking behaviour. Our findings are in agreement with earlier findings of Savory et al. (1999), who found TRP supplementation to decrease feather pecking damage in growing bantams at 4 and 6 weeks of age, suggesting a decrease in severe feather pecking. In our study, TRP supplementation did not significantly decrease severe feather pecking. However, severe feather pecks were mostly embedded in bouts of gentle feather pecking, suggesting a common underlying motivation and neurobiological system (Riedstra and Groothuis, 2002).

Savory et al. (1999) suggested that sedative-like properties of TRP were responsible for the decrease in feather pecking (damage). In our study however, chronic TRP treatment did not result in sedation of the birds, as HFP chicks even became more active. This difference might be explained from the difference in experimental set-up. In the study of Savory, from day 1 of age, chicks were given supplemental TRP and were raised without access to litter. However, in the present study, supplemental TRP was provided and litter was removed, at a later developmental stage of the chicks. Possibly, a longer administration of high dietary TRP levels, may lead to sedation of the birds. In future studies such effects of chronic TRP supplementation should be investigated.

Supplementary TRP also affected other behavioural elements. In the HFP line, an increase in both feeding and walking, was accompanied by a decrease in preening behaviour. In the LFP line TRP treatment decreased foraging, and also increased feeding behaviour. The

increase in the duration of feeding behaviour in both lines, might be explained from the role of the 5-HT system in the regulation of hunger and satiation. However, most studies report of a decrease in feed intake in response to TRP treatment (Lacy et al., 1986; Rosebrough, 1996). Six days of TRP administration to commercial layer breeder hens at a level of 5 g/kg has been reported to end the incidence of hysteria and stimulate feed consumption (Laycock and Ball, 1990). However, in the present study, only the time spent with the head in the feeding trough, and not the actual feed intake was measured. We did not observe an effect of TRP on bodyweight (data not shown), suggesting no effect on feed intake. However, current results are hard to interpret and the effect of TRP on (duration of) feeding should be investigated further.

Recently (van Hierden et al., 2004), we suggested that feather pecking behaviour might be a suitable animal model for obsessive compulsive disorder (OCD), as feather pecking has clear compulsive characteristics. Once birds start feather pecking, they tend to do so successively and their pecking behaviour is difficult to discourage. Furthermore, impaired 5-HT neurotransmission is implicated in obsessive compulsive disorders (for a review see Blier and de Montigny, 1998)as well as feather pecking (van Hierden et al., 2004). Chronic enhancement of 5-HT neurotransmission via blocking of 5-HT reuptake with selective serotonin reuptake inhibitors (SSRIs), alleviates OCD symptoms in most cases (Bergqvist et al., 1999; Blier and Montigny de, 1999). In future studies it should be investigated whether chronic treatment with SSRIs decreases the performance of feather pecking behaviour.

Furthermore, it cannot be excluded that serotonin exerts it actions via the dopaminergic system. Kjaer et al. (2002) showed that acute haloperidol treatment, significantly reduced feather pecking behaviour in adult laying hens. Haloperidol, a drug with anti-OCD effects, is a $D_2$ receptor antagonist which, with acute administration, increases DA-turnover by blocking the presynaptic DA autoreceptor (McElvain and Schenk, 1992). This suggests that low DA neurotransmission might be involved in the performance of feather pecking behaviour. In future studies the relationship between the serotonergic and dopaminergic system in relationship to feather pecking should be investigated.

### *4.3. Summary and conclusion*

Results of the present study confirm our hypothesis that feather pecking behaviour is triggered by low serotonergic neurotransmission, as increasing serotonergic tone, by increasing dietary TRP, decreases gentle feather pecking behaviour.

In conclusion, differences in the sensitivity for the development of feather pecking are associated with a difference in serotonergic function. In the future, more pharmacological experiments, studying the role of serotonergic or other neurobiological systems (e.g. dopaminergic system) are necessary to reveal the exact mechanisms underlying feather pecking behaviour.

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